Supplemental Data

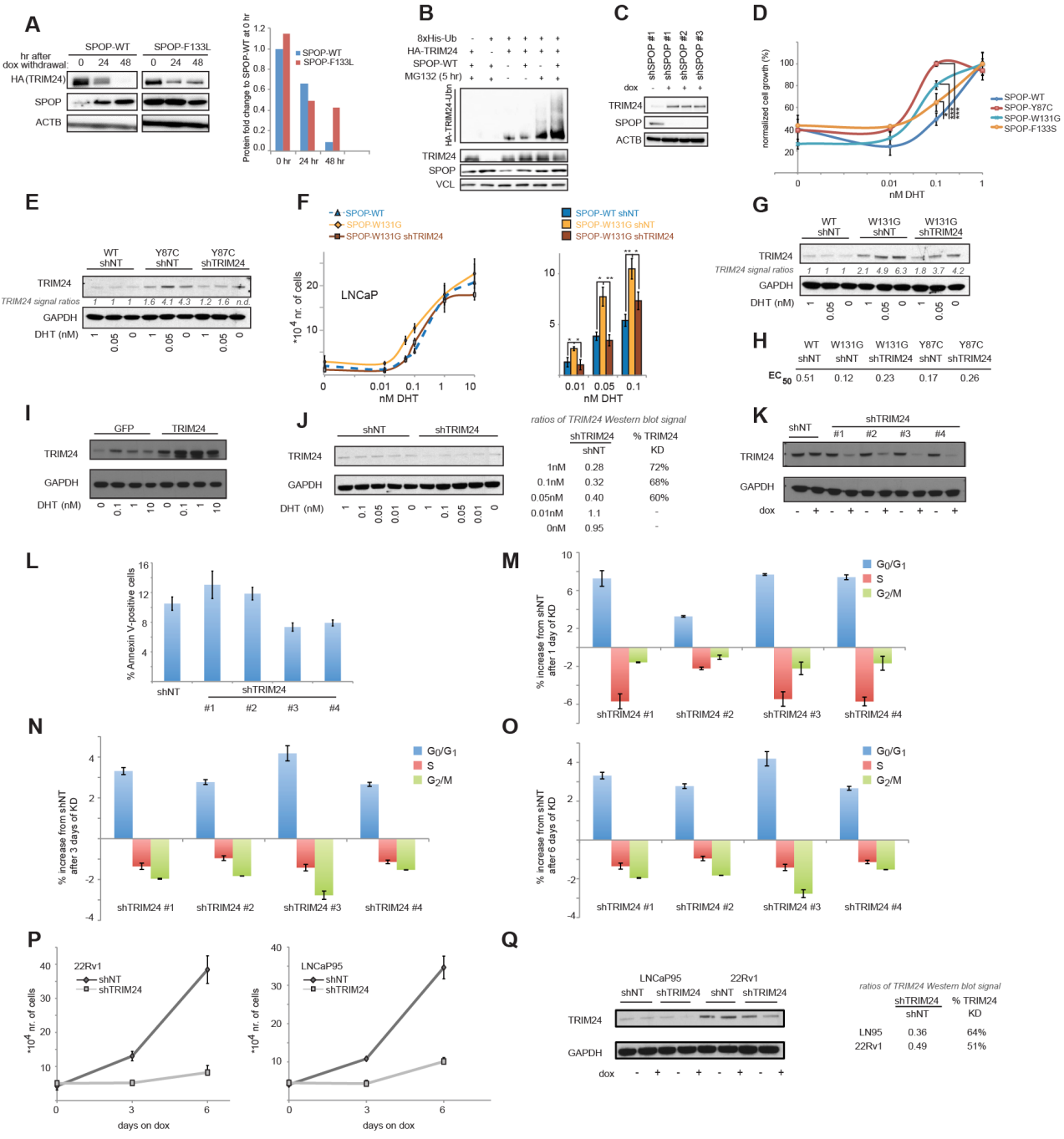

Figure S1 (related to Figure 1).

(A) LNCaP cells harboring doxycycline (dox)-inducible HA-tagged TRIM24 were engineered to over-express either SPOP wild-type (WT) or SPOP-F133L. (Left) HA-TRIM24 protein decay was assessed by Western blot after dox withdrawal at the indicated time points in both cell lines. (Right) HA-TRIM24 protein levels were quantified and plotted. Actin (ACTB) was used as a loading control. **(B)** 293T cells were transfected with 8xHis-Ubiquitin (Ub) and indicated constructs in the presence (+) or absence (-) of 10μM MG132. Western blots of protein lysates and 8xHis-Ub pull downs using nickel beads were run and blotted for the indicated factors. Vinculin (VCL) was used as a loading control. **(C)** LNCaP cells harboring three independent dox-inducible shRNAs targeting SPOP for knock-down (KD) were cultured with or without dox and TRIM24 protein levels were assessed by Western blot. ACTB was used as a loading control. **(D)** LNCaP cells stably expressing SPOP-WT, SPOP-Y87C, SPOP-W131G or SPOP-F133S were grown for 6 days and counted by hemacytometer (n=3) at the indicated concentration of DHT. Each cell line was normalized to the maximal cell number reached (100%) and the resulting normalized growth values were plotted against a $\log_{10}$ scale of the respective DHT concentration. Representative Western blots can be found in Figure 1A. Statistical analysis was performed using a one-tailed student's t-test assuming equal variance * $p<0.05$, *** $p<0.005$. **(E)** LNCaP cells stably expressing SPOP-WT (WT) or SPOP-Y87C (Y87C) were additionally infected with lentiviral, dox-inducible shRNA targeting either TRIM24 or a non-targeting (NT) control. Cells were treated with dox and grown for 6 days at the indicated concentration of DHT. TRIM24 protein levels were measured by Western blotting. GAPDH served as a loading control. TRIM24 Western blot signals were quantified and values detected at WT shNT at 1nM, 0.05nM or 0nM DHT were set at 1 and the values detected at Y87C shNT and at Y87C shTRIM24 were expressed as a ratio thereof. Note that the signal for Y87C shTRIM24 at 0nM DHT could not be determined (n.d.). **(F)** LNCaP cells stably expressing SPOP-WT (WT) or SPOP-W131G (W131G) were additionally infected with lentiviral, dox-inducible shRNA targeting either TRIM24 or a NT control. Cells were treated with dox, grown for 6 days and counted as in (D) at the indicated concentration of DHT. (Left) Cell numbers were plotted against a $\log_{10}$ scale of the DHT concentration. (Right) Cell numbers under low DHT levels are additionally highlighted in a bar plot. Statistical analysis was performed using a two-tailed student's t-test assuming unequal variance * $p<0.05$, ** $p<0.01$. **(G)** TRIM24 protein levels were measured by Western blotting. GAPDH served as a loading control. TRIM24 Western blot signals were quantified and values detected at WT shNT at 1nM, 0.05nM or 0nM DHT were set at 1 and the values detected at W131G shNT and at W131G shTRIM24 were expressed as a ratio thereof. **(H)** Based on cell proliferation numbers under different DHT concentrations (nM), the DHT-induced half maximal effective concentration (EC_{50}) values of proliferation were calculated for the different SPOP cell lines. The "log agonist vs. response with 3 parameters using non-linear regression" method in the prism program was used. **(I)** LNCaP cells harboring dox-inducible WT *TRIM24* or *GFP* cDNA were treated with dox for 6 days and grown in the presence of the indicated concentration of DHT. TRIM24 protein levels were measured by Western blotting. GAPDH served as a loading control. **(J)** LNCaP cells stably expressing dox-inducible shRNA targeting TRIM24 or a NT control were treated with dox for 6 days and grown in the presence of the indicated concentration of DHT. TRIM24 protein levels were measured by Western blotting. GAPDH served as a loading control. TRIM24 Western blot signals were quantified and the KD levels of TRIM24 protein at 0.05, 0.1 and 1nM DHT were calculated. **(K)** LNCaP-abl cells stably expressing 4 different dox-inducible shRNAs targeting TRIM24 or a NT control were treated with PBS or dox at day 0 and were grown for 9 days. TRIM24 protein level in the presence (+dox) or absence (-dox) of shRNA-expression was measured by Western blotting. GAPDH served as a loading control. **(L)** Apoptotic LNCaP-abl cell levels were assessed by Annexin V staining followed by FACS analyses after 6 days of shRNA induction. **(M-O)** Levels of different cell cycle stages were measured by propidium iodide-based DNA staining followed by FACS analyses after a 1 day **(M)**, 3 day **(N)** or 6 day **(O)** shRNA-induced KD of TRIM24. The values are depicted for each shTRIM24-expressing line as changes in the cell cycle profile compared to shNT. Data are represented as mean +/- SEM. **(P)** CWR-22Rv1 (22Rv1) and LNCaP95 cells stably expressing dox-inducible shRNA targeting TRIM24 or a NT control were treated with dox and grown for 6 days. Cell numbers were counted by hemacytometer (n=3) after 0, 3 and 6 days and values were plotted. 22Rv1 cells were cultured in full media, whereas LNCaP95 cells were cultured in serum-starved media. Statistical analysis was performed using a two-tailed student's t-test assuming unequal variance *** $p<0.005$. **(Q)** TRIM24 protein levels in the presence (+dox) or absence (-dox) of shRNA-expression were measured by Western blotting. GAPDH served as a loading control. TRIM24 Western blot signals were quantified and the KD level of TRIM24 protein in the presence of shRNA-expression was calculated for each cell line. Data in proliferation curves is represented as mean +/- SEM.

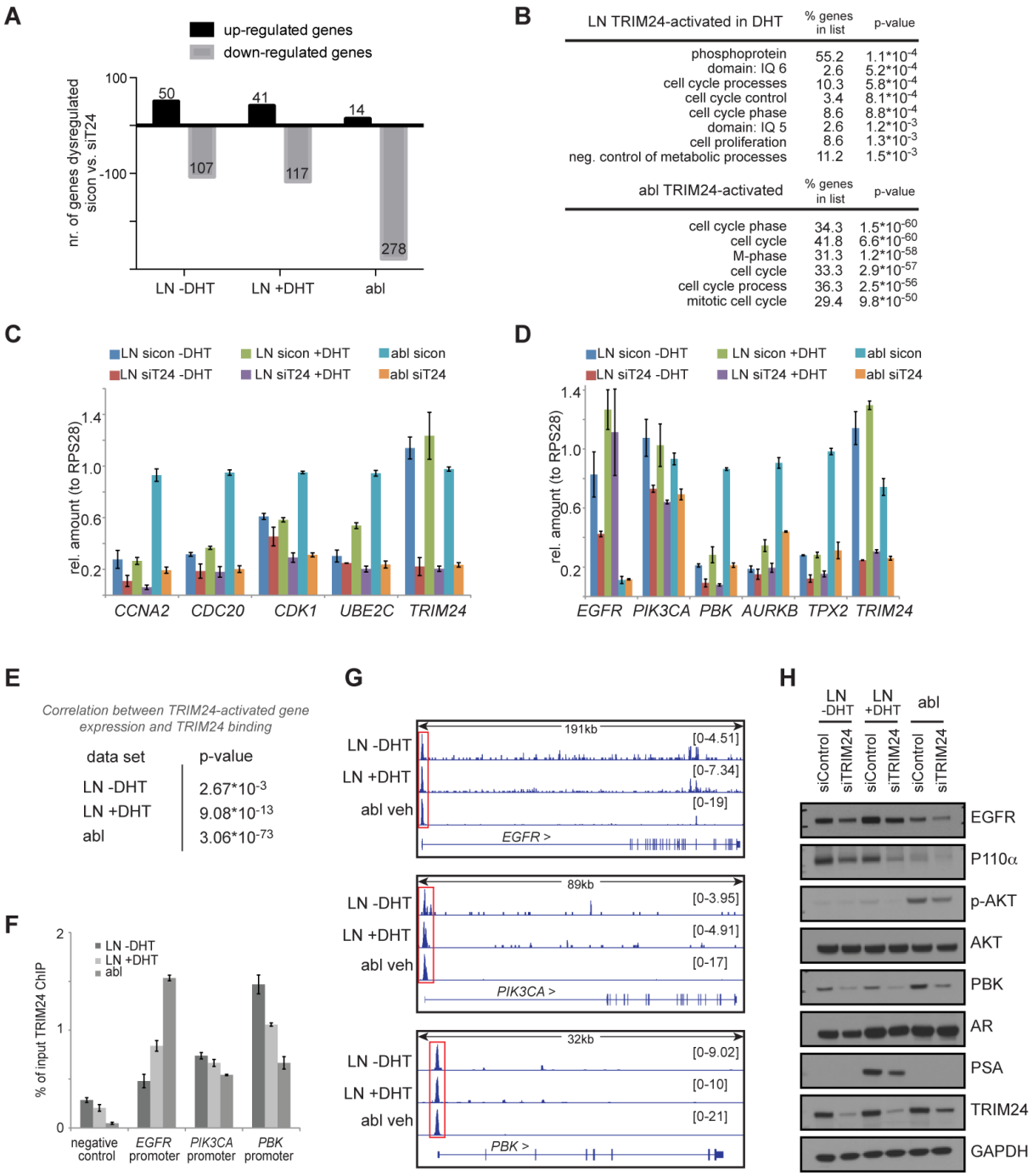

Figure S2 (related to Figure 2).

(A) Bar graph depicting the number of up- and down-regulated genes upon TRIM24 depletion from microarray analyses (SAM, fold-change >2). LNCaP (LN) cells were transfected with either si targeting control (sicon) or targeting TRIM24 (siT24) for 48 hr and grown in the absence (-DHT) or presence (+DHT) of 10nM DHT for the last 24 hr. LNCaP-abl (abl) cells were cultured under hormone-starvation and transfected with either sicon or siT24 for 48 hr. **(B)** Functional annotation of genes activated by TRIM24 was performed with DAVID gene ontology queries, where the most significantly enriched terms are depicted (Huang da et al., 2009). **(C, D)** Effects of control or TRIM24 knock-down on genes involved in cell proliferation in the different data sets were validated by quantitative PCR (qPCR) analysis (n=3). The experiments show **(C)** cell cycle genes that are up-regulated in CRPC and **(D)** direct TRIM24 targets. **(E)** Binding and Expression Target Analysis (BETA) (Wang et al., 2013) was performed to correlate TRIM24-regulated gene expression with TRIM24 binding. Significant p-values for TRIM24

acting as a transcriptional activator are depicted. **(F)** TRIM24-specific ChIP followed by qPCR (n=2) was performed in LN cells treated with 10nM DHT (+DHT) or vehicle (-DHT) for 4 hr, and in the CRPC line abl. **(G)** Screen shots depicting the signal traces from TRIM24-specific ChIP-seq experiments at the *EGFR*, *PIK3CA* and *PBK* gene loci. The minimal and maximal values of the respective traces are indicated in parenthesis on the right. **(H)** Western blot analyses of the indicated proteins and pS473-AKT (p-AKT). LN and abl cells were transfected with either sicon or siT24 and cultured as indicated in (A). qPCR results are represented as mean +/- SEM.

Table S1 (related to Figure 2), provided as separate Excel file. TRIM24 cistrome motif analysis.

Table S2 (related to Figure 2), provided as separate Excel file. TRIM24 target gene lists.

Table S3 (related to Figure 2), provided as separate Excel file. TRIM24 target pathway analyses.

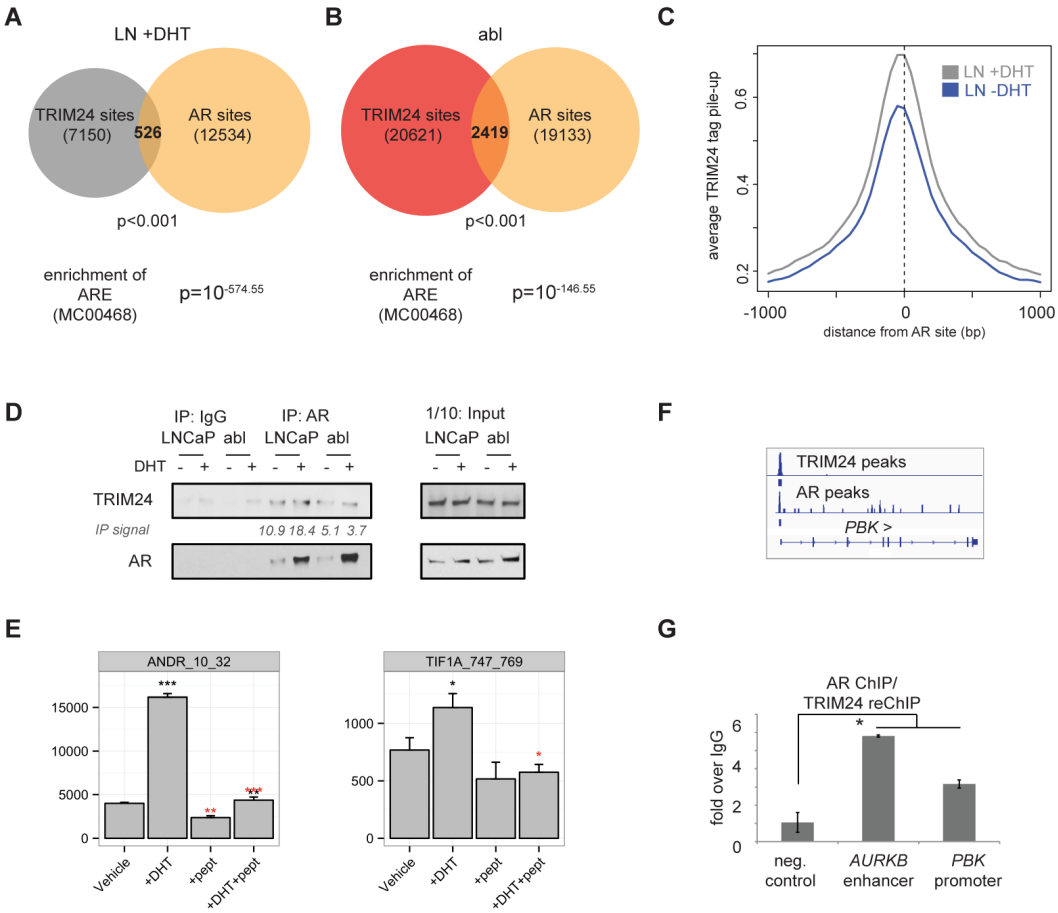

Figure S3 (related to Figure 3).

(A, B) Venn diagrams overlapping TRIM24 and AR genomic binding reveal **(A)** a 526 site overlap in LNCaP (LN) cells treated with 10nM DHT (+DHT) for 4 hr ($p<0.001$, random permutation), and **(B)** a 2419 site overlap in LNCaP-abl (abl) cells ($p<0.001$, random permutation). The total number of TRIM24-specific genomic binding sites in each data set is indicated in parenthesis. The androgen response element (ARE, MC00468) is significantly over-represented in the AR/TRIM24 overlapping sites. The respective p-values were determined with SeqPos (He et al., 2010) for sites present in (A, B). **(C)** SitePro analysis (Shin et al., 2009) of the composite TRIM24 ChIP-seq signals at AR sites (from A) determined in LN cells in the presence (+DHT) or absence (-DHT) of a 4 hr 10nM DHT stimulation shows a DHT-responsive enrichment of TRIM24 at AR sites. AR ChIP-seq data sets used in the analyses (A-C) were previously published by the Brown lab and others (Sahu et al., 2011). **(D)** Endogenous AR was immunoprecipitated (IP) from nuclear extracts and blotted for endogenous TRIM24 in LNCaP as well as in abl cells cultured in the presence (+) or the absence (-) of a 4 hr 10nM DHT treatment. TRIM24 Western blot signals were quantified. Signals from the IgG-specific control IP were subtracted from the AR IP signal to determine the level of TRIM24 signal above background (IP signal). The respective values are depicted. **(E)** MARCoNI peptide arrays (n=3, PamGene) containing nuclear receptor coregulator-derived motifs were used to assess the interaction between the AR ligand binding domain (LBD) and the positive control AR peptide (aa 10-32, ANDR_10_32) or the TRIM24 LxxLL motif-containing peptide (aa 747-769, TIF1A_747_769). We confirmed a significant interaction between the TRIM24 peptide and the AR LBD, which increased upon DHT addition (black asterisks, * $p<0.05$) and decreased after the addition of competing AR FxxLF peptides (pept) in solution (red asterisks, * $p<0.05$). Statistical analysis was performed using a two-tailed student's t-test assuming unequal variance * $p<0.05$, ** $p<0.01$, *** $p<0.005$. **(F)** Screenshot depicting the signal traces of TRIM24- and AR-specific ChIP-seq experiments in abl cells at the *PBK* gene. **(G)** AR-directed ChIP followed by a TRIM24-directed ChIP and quantitative PCR in abl cells showed significant enrichment of both factors at the *PBK* promoter and the *AURKB* enhancer, both from the 21-gene signature, when compared to an IgG-directed background control (n=2). Statistical analysis was performed using a one-tailed student's t-test assuming equal variance * $p<0.05$. Data are represented as mean +/- SEM.

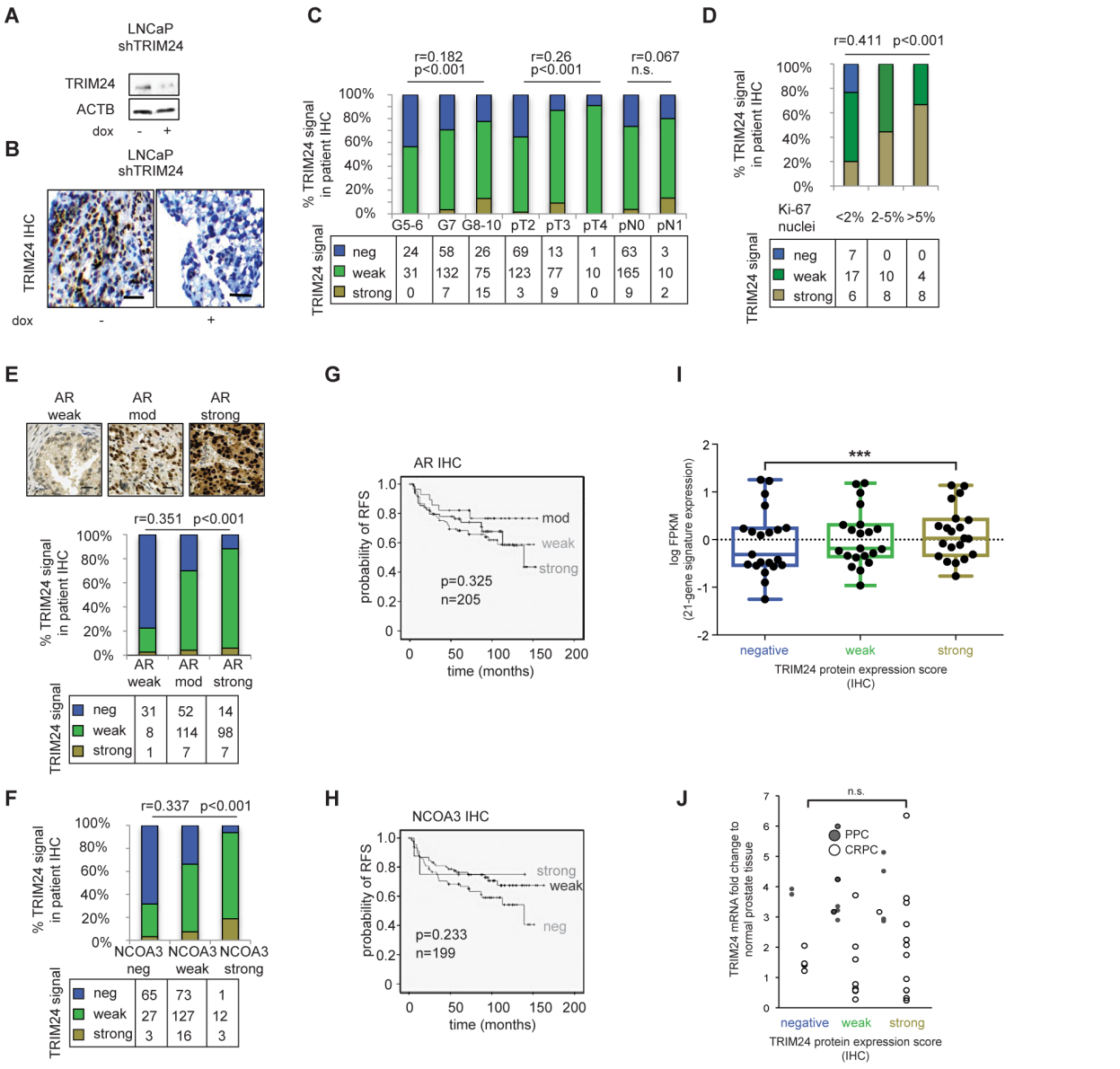

Figure S4 (related to Figure 4).

(A, B) LNCaP cells stably expressing doxycycline (dox)-inducible shRNA targeting TRIM24 were grown in the presence (+dox) or absence (-dox) of shRNA induction. **(A)** TRIM24 protein levels were measured by Western blotting. Actin (ACTB) was used as a loading control. **(B)** The indicated LNCaP lines were formalin-fixed and paraffin-embedded and subjected to immunohistochemistry (IHC) to assess the specificity of the TRIM24 antibody used for IHC analyses in patient tissue. Bar represents 80 μm. **(C)** Correlation of negative (neg), weak and strong TRIM24 protein nuclear staining with combined histological Gleason tumor grade (G), tumor size (pT), and metastatic spread to local lymph nodes (pN). **(D)** Correlation of neg, weak and strong TRIM24 protein nuclear staining with percentage of nuclear Ki-67 positivity. Ki-67 positive nuclei were categorized as follows: <2%, 2-5% and >5% positive nuclei. **(E, F)** Correlation of TRIM24 protein nuclear staining with **(E)** AR and **(F)** NCOA3 staining by IHC using a three-tiered scoring system. In addition, (E) shows a representative AR staining with a scale bar of 50 μm. **(G, H)** Kaplan-Meier curves for PSA-based recurrence free survival (RFS). Patients were stratified according to the **(G)** AR and **(H)** NCOA3 IHC signal (p-value, log rank test). **(I)** The expression levels of the 21 genes co-activated by AR and TRIM24 were assessed by RNA-seq in an independent cohort of PPC and matched normal tissues. The respective values were individually plotted as the log of their FPKM values. TRIM24 protein

levels were also assayed by IHC and the expression values were plotted against the TRIM24 IHC score (negative, weak, strong). The average FPKM values of the 21 genes, together with the TRIM24 IHC score can be found in Table S4. Statistical analysis was performed with the non-parametric Friedman test and a positive correlation between expression of the 21 genes and TRIM24 protein levels was found. p=0.0006 (*** p<0.005). **(J)** 12 primary and 13 castration-resistant tumors were assessed for TRIM24 protein levels by IHC and *TRIM24* mRNA levels by quantitative PCR. mRNA levels were normalized to the mean *TRIM24* expression level of 8 normal prostate tissues analyzed in parallel. There is no statistical correlation between protein and mRNA level pairs (R=0.13, Spearman rank test).

Table S4 (related to Figure 4), provided as separate Excel file. Tumor RNA-seq FPKM values.

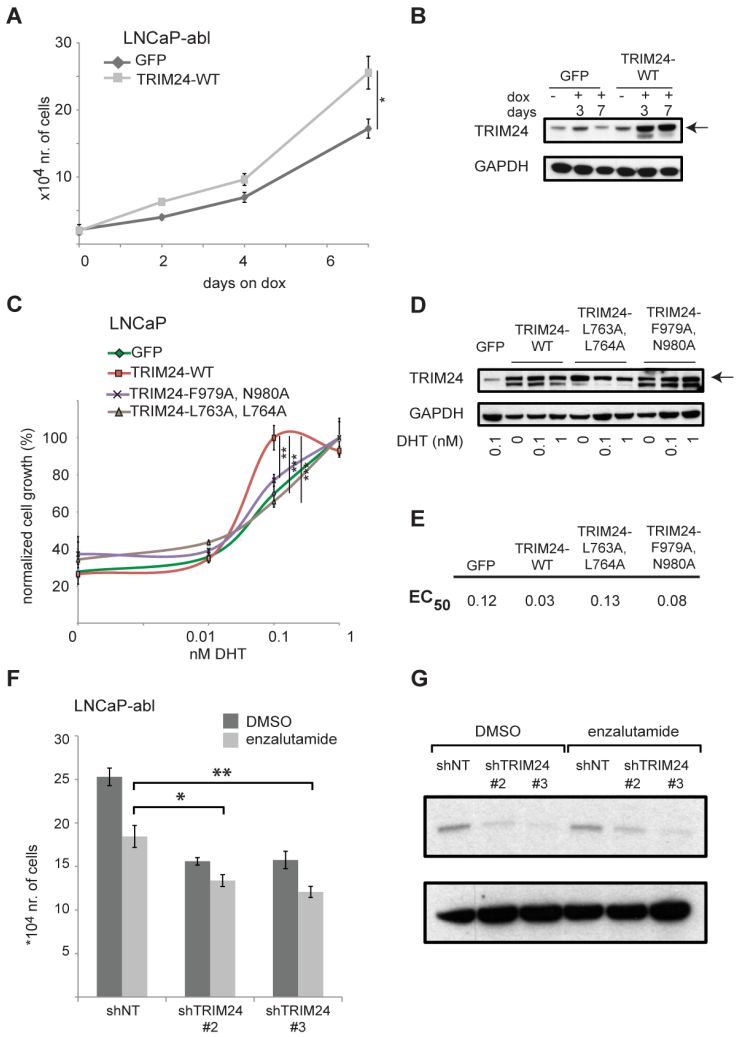

Figure S5 (related to Figure 7).

(A, B) LNCaP-abl (abl) cells harboring doxycycline (dox)-inducible GFP or TRIM24 wild-type (WT) were grown in the presence of dox for 7 days. **(A)** Abl cell numbers were counted after the indicated days and plotted. Statistical analysis was performed using a two-tailed student's t-test * $p<0.05$. **(B)** Protein levels of TRIM24 were assessed by Western blotting after 0, 3 or 7 days of dox exposure. The full-length form of TRIM24 is indicated with an arrow. GAPDH served as a loading control. Statistical analysis was performed using a two-tailed student's t-test * $p<0.05$. **(C-E)** LNCaP cells harboring dox-inducible GFP, TRIM24-WT, TRIM24-L763A, L764A or TRIM24-F979A, N980A were treated with dox for 6 days and grown in the presence of the indicated concentration of DHT. **(C)** Cell numbers were counted by hemacytometer (n=3) and divided by the largest value reached in each individual cell line. The resulting normalized growth values were plotted against a $\log_{10}$ scale of the respective DHT concentration. Statistical analysis was performed using a one-tailed student's t-test ** $p<0.01$, *** $p<0.005$. **(D)** TRIM24 protein levels were measured by Western blotting and full-length TRIM24 is indicated with an arrow. GAPDH served as a loading control. **(E)** Based on the proliferation numbers under different DHT concentrations (nM), the DHT-induced half maximal effective concentration (EC_{50}) values of proliferation were calculated for the different LNCaP cell lines. The "log agonist vs. response with 3 parameters using non-linear regression" method in prism was used. **(F)** Abl cells stably expressing two different dox-inducible shRNAs targeting TRIM24 or a non-targeting (NT) control were treated with DMSO or 10μM of the AR antagonist enzalutamide for 6 days. Cells were additionally treated with dox during this time and cell numbers were counted by hemacytometer (n=3) and values were plotted. Statistical analysis was performed using a two-tailed student's t-test assuming unequal variance * $p<0.05$, ** $p<0.01$. **(G)** TRIM24 protein levels were measured by Western blotting. GAPDH served as a loading control. Data points are represented as mean +/- SEM.

Supplemental Experimental Procedures:

Cell culture and proliferation assays

LNCaP, LNCaP-abl (abl), LNCaP95 (Hu et al., 2012) and CWR-22Rv1 (22Rv1) cells were cultured at 37°C at 5% CO_2 in RPMI 1640 medium with (LNCaP, 22Rv1) and without (abl, LNCaP95) phenol red. The medium was supplemented with 10% FBS (LNCaP, 22Rv1) or 10% charcoal-dextran-treated FBS (abl, LNCaP95), penicillin (100u/mL), streptomycin (100u/mL) and L-glutamine (2mM). Cells were genotyped by bioSYNTHESIS and were regularly tested for mycoplasma contamination.

For proliferation assays the prostate cancer cells were plated in 24 well plates (4-5*10^4 cells/well). At indicated time points, the cells were trypsinized and collected. The number of viable cells was determined by Trypan blue exclusion staining and directly assessed with a hemacytometer using independent triplicates.

Lentiviral vectors

Lentiviral particles were produced as previously described (Barde et al., 2010). Viral infections were performed using unconcentrated virus. Infected cells were stably selected using puromycin (SPOP, pTRIPZ, pTRE series) or blasticidin (pLCW series) at following concentrations: puromycin at 1ug/mL and 2ug/mL for LNCaP and abl cells, respectively; blasticidin at 2ug/mL for both cell lines.

The SPOP-expressing lentiviral (LV) vector plasmids were previously described. Dox-inducible shRNA constructs from the LV.pTRIPZ plasmid series targeting either control

non-targeting (shNT) or TRIM24 (shTRIM24) were purchased from Openbiosystems and the target sequences are listed below. CMV-driven and dox-inducible cDNA constructs for the LV.pLCW or the LV.pTRE plasmid series, respectively, were constructed using the Gateway system. Briefly, pENTRY vectors containing TRIM24 or GFP cDNAs were made by PCR using GFP (ID 12252), TRIM24-WT (ID 28138) or TRIM24-F979A, N980A (ID 28136) expressing plasmids as templates (all obtained from Addgene, see ID). The TRIM24-L763A, L764A plasmid was generated by site-directed mutagenesis (Stratagene XL QuickChange mutagenesis kit) on pENTRY.TRIM24-WT. Primers are specified below. Recombination reactions between the pENTRY vectors and the pLCW destination LV vector (kindly provided by Benjamin Yazdanpanah) (Pongratz et al., 2010) or the pTRE destination LV vector (kindly provided by Andrea Corsinotti) (Gubelmann et al., 2014) were performed following the Gateway cloning protocol (Invitrogen).

Protein procedures and Western blot quantifications

For Western blot analyis, cells were lysed in 50mM Tris-HCl pH 7.5, 150mM NaCl, 1mM EDTA, 1mM EGTA, 0.5% NP-40, 1% Triton X-100 supplemented with protease inhibitors and subjected to SDS-PAGE. Antibodies used were: EGFR (D38B1), PI3K P110α (C73F8), AKT (9272), AKT p-Ser473 (9271), PSA/KLK3 (D6B1), PBK/TOPK (4942) all from Cell Signaling Technologies. AR (N-20), TRIM24/TIF1α (C-4), GAPDH (6C5) all from Santa Cruz.

Endogenous co-IP was carried out as previously described (Ni et al., 2013) with the following changes. After nuclear lysis and over-night incubation with anti-AR (N-20) or anti-IgG, protein complexes were precipitated with magnetic beads. Beads were then washed, and complexes were eluted by boiling in Laemmli sample buffer (Bio-Rad).

The MARCoNI peptide array (PamGene) was described (Houtman et al., 2012) with following modifications. We used recombinant AR LBD (Invitrogen PV4388) in TR-FRET coregulator buffer A (Invitrogen PV4384).

We systematically quantified Western blots showing TRIM24 protein knock-down or over-expression. More specifically, Western blot bands were quantified by using the "Segmented line" and "Plot Profile" tools in the Image J program. Resulting curves were analyzed using the R programming language. Individual bands were identified by using a spline interpolation. Single values were then assigned to bands calculating the mean value for each band. In some cases where the mean value was inadequate, the maximum value of the interpolation was used instead. Fold changes were calculated after measuring and subtracting the background grey levels of the bands.

Gene expression and microarray analysis

Hormone-depleted LNCaP and abl cells were transfected with either control siRNA (sicontrol) or siRNA targeting TRIM24 (siTRIM24), and LNCaP cells were then treated with 10nM DHT or vehicle for 24 hr, while abl cells were grown with vehicle only. For siRNA target sequences see below. 48 hr after siRNA transfection, total RNA was

isolated using the RNeasy plus kit (Qiagen). Quantitative RT-PCR (qPCR) to measure single gene expression was performed as outlined in (Groner et al., 2010) using primers described below. For microarray analyses the RNA was hybridized to Affymetrix human U133 plus 2.0 expression arrays (Affymetrix) at the Dana-Farber Cancer Institute Microarray Core Facility. Gene expression data were normalized and log-scaled using the RMA algorithm (Irizarry et al., 2003) and the RefSeq probe definition (Dai et al., 2005). Differentially expressed genes were determined by using either the linear models for microarray data algorithm LIMMA (Smyth, 2004) or the Significance analysis of microarrays algorithm SAM (Tusher et al., 2001). Microarray data have been deposited at the GEO repository under the accession number GSE69330. Additional published gene expression data used for analyses can be found in GSE11428.

Gene Set Enrichment Analysis (GSEA) was carried out as follows: Significantly represented biological pathways or processes in specific gene lists were identified by querying against a curated set of genes through the MSigDB database. In addition, comparisons between ranked gene expression data sets and specific gene lists were performed with the GSEA analysis tool, where normalized enrichment scores and the significance of enrichment are calculated.

Chromatin Immunoprecipitation (ChIP), ChIP-reChIP, ChIP-seq

LNCaP cells were starved for 72 hr and then treated with DHT or vehicle for 4 hr, whereas abl cells were constantly kept under hormone-starved conditions. Thereafter, ChIP was performed. Briefly, 1-2x10^7 cells were cross-linked with 1% formaldehyde at

RT for 10min and quenched with glycine. Chromatin was extracted in ChIP buffer (0.1%SDS, 1% Triton X-100, 10mM Tris-HCl pH7.4, 1mM EDTA, 0.1% NaDOC, 0.25% sarcosyl, 1mM DTT, protease inhibitors) and sonicated to an average fragment size of 1kb using the bioruptor water bath sonicator (Diagenode). Chromatin-antibody complexes were incubated over night with the following antibodies anti-TRIM24 (A300-815A, Bethyl), anti-AR (N20, Santa Cruz) and rabbit IgG (Santa Cruz) as a control and captured with Dynabeads Protein A (Invitrogen). After RNase A and Proteinase K treatment, ChIP DNA was quantified by Quant-iT dsDNA HS assay (Invitrogen).

For ChIP-reChIP, we used the same protocol, but after the initial round of overnight IP (with AR), we eluted the magnetic beads with 10mM DTT for 30 min at 37°C. The DTT was quenched with 0.3M NaCl containing ChIP buffer and free chromatin complexes were then subjected to a second round of IP using a different antibody (TRIM24 or IgG).

For targeted ChIP, extracted DNA was subjected to qPCR as outlined in (Groner et al., 2010) with specific primers.

For ChIP-seq, sequencing libraries were generated from 0.1-10ng ChIP or input DNA. They were ligated with specific primers and amplified according to the manufacturer's instructions (ThruPLEX-FD Prep Kit, Rubicon Genomics). Standard Illumina indexes (6bp) were used and the libraries were pooled and sequenced on the Illumina Hiseq-2000 platform at the Dana-Farber Cancer Institute Center for Cancer Genome Discovery. ChIP-seq reads were aligned to the hg19 genome assembly using Bowtie

(Langmead et al., 2009) and ChIP-seq peaks were called using MACS 2.0 (Zhang et al., 2008). Regions of enrichment comparing ChIP and input control signal exceeding $q<0.01$ were called as peaks. Files containing raw reads and peak-containing bed files have been deposited at the GEO repository under the accession number GSE69331.

Correlation between TRIM24-activated gene expression and binding of TRIM24 was performed with the Binding and Expression Target Analysis (BETA) basic algorithm (Wang et al., 2013).

Overlap significance was calculated based on 10^4 random permutations. In each permutation, peaks from the TRIM24 ChIP-seq data sets were randomly shuffled through the whole hg19 genome, and then count overlaps with the specific peaks in the respective AR cistromes or H3K27-acetyl cistromes were calculated After 10^4 permutations, we determined the significance of the mean value of empirical random distribution of overlapped peaks ($p<0.001$).

In Vivo Ubiquitylation

For in vivo ubiquitylation $2.4x10^6$ 293T cells were seeded and transiently transfected the next day with the following plasmids: LV.pTRE-TRIM24 plasmid (2 µg), pCW107-SPOP-WT (2 µg), and CMV-8xHis-Ub (2 µg). Empty vector pCW107 was used to bring the total plasmid DNA to 6 µg for each transfection. Two days later HA-TRIM24 was induced by doxycycline and after three days cells were treated with MG132 (10 µM) or DMSO for an additional 5 hours. The cells were then washed twice with ice cold PBS,

scraped off the plates in PBS and then collected by centrifugation. A small aliquot of the cells was lysed in RIPA buffer, and the rest were lysed in Buffer C (6M guanidine-HCL, 0.1M Na2HPO4/NaH2PO4, 10 mM imidazole, pH 8.0). After sonication, the whole cell extract were mixed with 100 µL of Ni-NTA agarose magnetic beads at 4°C overnight. Next, the Ni-NTA beads were washed twice with Buffer C, twice with Buffer D (1 Volume of Buffer C: 3 volumes of Buffer E), and once with Buffer E (25 mM Tris.CL, 20 mM imidazole, pH 6.8). Bound proteins were then eluted by boiling in 1x SDS loading buffer containing 300 mM imidazole, resolved by SDS polyacrylamide gel electrophoresis, and detected by immunoblot analysis.

Recurrence-free Survival Analysis based on gene signature

R-based scripts were used to assess prostate cancer patient gene expression data from the Taylor data set (GSE21034). Gene-specific Z-score values were determined for patients with primary or metastatic disease. Values for genes present in our gene signature were extracted from the expression file. The resulting matrix of expression Z-scores was hierarchically clustered using the Ward method and Euclidian distance and displayed using the heatmap.2 function. Clinical data was matched to the samples in the clusters and Kaplan-Meier plots were calculated using the survfit function. Differences between clusters are calculated using the survdif function and resulting Chi^2 values were converted to p-values with pchisq. The probability of recurrence was calculated by using biochemical recurrence measurements defined as surge in PSA after treatment as defined in the Taylor data set.

Human Tumor Samples

We previously characterized TRIM24 protein expression in primary prostate tumors in tissue microarrays composed of paraffin-embedded prostate tissue cores from two different pathology institutes. Specimens were collected between 1993 and 2007 from the Institute of Surgical Pathology, University of Zurich, Switzerland, and the Institute of Pathology, University of Regensburg, Germany. These tissue microarrays include a limited number of samples of hyperplastic prostate tissue and advanced, castration-resistant tumors. Comprehensive clinical data including patient's age, BMI, combined Gleason score, tumor size, local metastatic spread to local lymph nodes, resection status, PSA levels at diagnosis, and biochemical recurrence-free survival was available as well. SPOP mutation status was available for a subset of tumors as described earlier. The local scientific ethics committees approved both cohorts (approval no: StV-Nr. 25/2007).

In order to analyze TRIM24 protein expression in a larger number of patient samples with castration-resistant disease, we constructed tissue microarrays containing 82 formalin-fixed, paraffin-embedded, castration-resistant prostate cancer tissues. The specimens were collected between 1998 and 2013 from the Institute of Surgical Pathology, University of Zurich, Switzerland, and included a series of 67 consecutive (nonselected) palliative transurethral resected prostatic tissue samples, 13 bone metastases, one lymph node metastasis, one brain metastasis, and one lung metastasis. Hematoxylin and eosin–stained slides of all specimens were re-evaluated by one experienced pathologist (P.J.W.) to identify representative areas. Only patients

with castration-resistant disease according to the guidelines of the prostate clinical trials working group were included (Scher et al., 2008). Patients were treated prior development of castration-resistance by orchiectomy (n=27), LHRH agonist (n=26), or bicalutamide (n=1). A subset of patients (n=28) got more than one treatment. Patients had an average age of 74 years (range: 56-87). The local scientific ethics committees approved the cohort (approval no. KEK StV 25-2008).

A validation cohort of sixty primary prostate cancer and matched normal prostatic tissue were collected from 2008 to 2012 at the Institute of Surgical Pathology at the University Hospital Zürich, Switzerland (approval no. KEK StV 25-2008). This cohort was used to correlate TRIM24 protein expression determined by IHC and tumor cell proliferation measured by percentage of Ki-67 positive tumor cells. In parallel, RNA was extracted from corresponding fresh-frozen tissue for transcriptome analysis.

Global Transcriptome Analysis on Primary Prostate Cancer Cohort

Tissue punches (1mm diameter) were taken from fresh frozen human prostatectomy samples of sixty primary prostate cancer and matched normal prostatic tissue were collected from 2008 to 2012 at the Institute of Surgical Pathology at the University Hospital Zürich, Switzerland (see above). Each tissue punches was homogenized in 200ul ice-cold homogenization solution (Promega) in the TissueLyser (Qiagen) for 45s at 30Hz with 5mm stainless steel beads. Total RNA was extracted using the Maxwell 16 LEV simply RNA Tissue Kit (Promega) according to the manufacturer's instructions. RNA integrity was assessed with a Bioanalyzer 2100 instrument (Agilent Technologies).

Only high-quality RNA (RNA integrity number >7) was used for sequencing.

Illumina library preparation for whole-transcriptome analysis was carried out at the Functional Genomics Center, Zurich, Switzerland, according to the manufacturer's protocol. RNA sequencing was carried out using an Illumina Hiseq-2500 instrument. All libraries were subjected to 101 cycles of paired-end sequencing. The processing of fluorescent images into sequences, base calling, and quality value calculations was performed using the Illumina data processing pipeline (version 1.8.2). Raw reads were first cleaned by removing adapter sequences, trimming low quality ends, and filtering reads with low quality (phred quality <20). Resulting sequences were aligned to the human reference genome (build hg19) using the STAR aligner software, v.2.4.0j (Dobin et al., 2013) and gene level expression was calculated as FPKM values (Fragments Per Kilobase exon-model and per Million total reads) using cufflinks, v.2.2.1 (Trapnell et al., 2010).

Immunohistochemistry

The immunohistochemistry procedures and scoring system for TRIM24 and NCOA3 have been described previously. For the detection of AR, slides were analyzed with the Bond-III automated staining system (Leica) using manufacture reagents for the entire procedure. For antigen retrieval, slides were incubated for 60 min in Tris/Borat-buffer at 100°C. Thereafter, slides were incubated with a mouse monoclonal antibody against AR (clone F38.4.1 from BioGenex) at the dilution of 1:500. Detections were performed using the detection refine DAB kit (Leica). Immunohistochemical staining was evaluated as follows for AR: Faint staining in more than 30% of tumor cell nuclei was referred as

weak, 30% or more tumor cell with an intermediate staining pattern (hardly discernable nucleoli) as moderate, and more than 30% of nuclei strongly stained (invisible nucleoli) as strong.

Quantitative PCR experiments on patient samples

RNA was extracted out of paraffin-embedded tissue from 13 castration-resistant prostate cancer samples represented on the CRPC tissue microarray (see above) and from an independent set of normal prostate (n=8) and primary prostate cancers (n=12) using the Maxwell® 16 LEV RNA FFPE purification kit according to the manufacturer's recommendations. Primary prostate cancers were analyzed for TRIM24 protein expression by IHC. *TRIM24* and *ACTB* mRNA expression was measured using the iKAPA SYBR FAST One-Step qRT-PCR Kit with 10ng RNA input in a total volume of 20 microliter for each reaction. PCR reactions were carried out on a Roche Light Cycler Lc480. Primer sequences are specified below and were used at an annealing temperature of 60°C. *TRIM24* mRNA expression was normalized to *ACTB* levels within individual samples of primary and castration-resistant prostate cancers and then compared to the mean expression level of normal prostate tissues analyzed in parallel.

Statistical Analysis on patient samples

Kendall beta-tau was used to test for correlation of immunohistochemical stainings and Spearman rank test for correlation of continuous mRNA fold changes with protein expression score. Univariate Cox regression analysis was used to evaluate statistical association between clinicopathological/immunohistochemical/mutation data and recurrence-free survival. Recurrence-free survival curves were calculated using the Kaplan–Meier method with significance evaluated by 2-sided log-rank statistics. Patients were censored at the time of their last tumor-free clinical follow-up visit. Time to PSA recurrence (cutoff 0.1 ng/mL) was selected as clinical endpoint. Only patients with primary prostate cancer undergoing radical prostatectomy and reaching the PSA nadir (<0.1 ng/mL) postoperatively were used for survival analysis. A multivariate Cox regression model with and without stepwise reverse selection was adjusted, testing the independent prognostic relevance of TRIM24 immunoreactivity. The proportionality assumption for all variables was assessed with log-negative log survival distribution functions. SPSS version 17.0 (SPSS) was used for statistical analyses. $p < 0.05$ was considered significant.

siRNA/shRNA sequences

siTRIM24	MM-005387-03-0010, siGENOME SMART pool, Human TRIM24 (8805)
D-005387-06	GAACAUACCACGACAAGCA
D-005387-07	AGACUUAUCUAAACCAGAA
D-005387-22	CUUUAGUAAUCGAGGAUAA
D-005387-23	CUUUAUAGCAAACGACUGA
sicontrol	D-001206-14-20, siGENOME Non-Targeting siRNA Pool #2
TRIPZ inducible lentiviral human **TRIM24 shRNA**	RHS4740-EG8805
# 1 V2THS_255059	ATTGTTTAGAGAGTCCAGC
# 2 V2THS_254581	TATTATTTAAGCAACTGGC
# 3 V2THS_16660	TAGGTGTTAACTTAACAAG
# 4 V2THS_254928	ATTATCTTCTGATTCATTC
Non-targeting control	CTCGCTTGGGCGAGAGTAAG

Primer sequences

Mutagenesis primers	**forward**	**reverse**
TRIM24-L763A, L764A	GCATACTCACCTCCGCGGCCTTAAATAGCAGTCAGAGC	GCTCTGACTGCTATTTAAGGCCGCGGAGGTGAGTATGC
mRNA qPCR primers (cell lines)	**forward**	**reverse**
RPS28 (normalization)	CGATCCATCATCCGCAATG	AGCCAAGCTCAGCGCAAC
TRIM24	TGTGAAGGACACTACTGAGGTT	GCTCTGATACACGTCTTGCAG
ANAPC10	CTGATGAAAGCTATACTCCAAGCA	GGAACATGAATCCAGCCACT
CCNA2	CAGAAAACCATTGGTCCCTC	CACTCACTGGCTTTTCATCTTC

CDC20	CCTCTGGTCTCCCCATTAC	ATGTGTGACCTTTGAGTTCAG
CDK1	CCTAGTACTGCAATTCGGGAAATT	CCTGGAATCCTGCATAAGCAC
UBE2C	TGGTCTGCCCTGTATGATGT	AAAAGCTGTGGGGTTTTTCC
mRNA qPCR primers (tissue samples)		
ACTB (normalization)	AGAGCTACGAGCTGCCTGAC	AGCACTGTGTTGGCGTACAG
TRIM24	CAGCCACAAATGCCTAAGCAG	GTGTTGGGAACTTGGATAACTGG
ChIP qPCR primers	**forward**	**reverse**
KIAA0066 (negative control)	CTAGGAGGGTGGAGGTAGGG	GCCCCAAACAGGAGTAATGA
AURKB (enhancer)	CGGCGGTTTTGTTATTGG	CTCGGCCTCTGTGTTCGAT
EGFR (TSS)	CCCTGACTCCGTCCAGTATTG	CGCTGCTCCCCGAAGAG
PBK (TSS)	AGCTGCCTCTAGCACCAACAC	CAGGAGGGTTCGAATTGCAA
PIK3CA (TSS)	CCTCCTCGCCTCAATTTCG	ACAATCCCCCGGAAGCA

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